EpiQuik™ BRD2 Binding Activity/Inhibition Assay Kit (Colorimetric)

Base Catalog # P-4058

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiQuik™ BRD2 Binding Activity/Inhibition Assay Kit (Colorimetric) is a complete set of essential components which enables an experimenter to colorimetrically quantify BRD2 binding activity/inhibition. The EpiQuik™ BRD2 Binding Activity/Inhibition Assay Kit can be used with purified BRD2 proteins or nuclear extracts from fresh tissue or cultured cells from human and mouse. Nuclear extracts can be prepared using your own successful method. For your convenience and the best results, Epigentek offers a nuclear extraction kit (Cat # OP-0002) optimized for use with this kit. Nuclear extracts can be used immediately or stored at –80°C for future use. Purified BRD2 can be isolated from recombinant proteins or from cell/tissues.

Starting Materials: Input materials can be nuclear extracts or purified BRD2 protein. The amount of nuclear extracts for each assay can be 1 µg to 20 µg with an optimized range of 5-10 µg. The amount of purified protein can be 10 ng to 500 ng, depending on the purity of the protein.

Internal Control: A BRD2 protein is provided in this kit as a control for the quantification of BRD2 binding activity. Because BRD2 binding activity can vary from tissue to tissue, and from normal and diseased states, it is advised to run replicate samples to ensure that the signal generated is validated.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the strip wells. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com
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KIT CONTENTS

Component	48 Assays Cat. #P-4058-48	96 Assays Cat. #P-4058-96	Storage Upon Receipt
WB (10X Wash Buffer)	12 ml	25 ml	4°C
BRL (BRD2 Ligand, 100X)*	50 µl	100 µl	–20°C
BRS (Ligand Binding Solution)	5 ml	10 ml	4°C
BB (Blocking Buffer)	10 ml	20 ml	4°C
AB (Assay Buffer)	3 ml	6 ml	4°C
CA (Capture Antibody, 1000 µg/ml)*	5 µl	8 µl	4°C
DA (Detection Antibody, 200 µg/ml)*	6 µl	10 µl	–20°C
ES (Enhancer solution)*	6 µl	10 µl	–20°C
DS (Developing Solution)	6 ml	12 ml	4°C
SS (Stop Solution)	6 ml	11 ml	RT
BRD2 Control (200 µg/ml)*	8 µl	16 µl	–20°C
BRD2 Inhibitor (100 µM)*	20 µl	40 µl	–20°C
8-Well Assay Strips (With Frame)	6	12	4°C
User Guide	1	1	RT

**For maximum recovery of the products, centrifuge the original vial after thawing prior to opening the cap.*

SHIPPING & STORAGE

The kit is shipped in two parts: one part at ambient room temperature, and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store **BRL**, **DA**, **ES**, **BRD2 Control,** and **BRD2 Inhibitor** at –20°C away from light; (2) Store **WB**, **BRS**, **BB**, **AB**, **CA**, **DS**, and the **8-Well Assay Strips** at 4°C away from light; (3) Store **all other components** at room temperature. The kit is stable for up to 6 months from the shipment date, when stored properly.

*Note: (1) Check if wash buffer, **WB**, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved; (2) check if a blue color is present in **DS** (Developing Solution). If it is blue this indicates contamination of the solution and it should not be used. To avoid contamination, transfer the amount of **DS** required into a secondary container (tube or vial) before adding **DS** into the assay wells.*

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Adjustable pipette or multiple-channel pipette
- ☐ Multiple-channel pipette reservoirs
- ☐ Aerosol resistant pipette tips

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- ☐ Microplate reader capable of reading absorbance at 450 nm
- ☐ 1.5 ml microcentrifuge tubes
- ☐ Incubator for 37°C incubation
- ☐ Distilled water
- ☐ Nuclear extracts or purified BRD2 protein
- ☐ Parafilm M or aluminum foil

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the EpiQuik™ BRD2 Binding Activity/Inhibition Assay Kit (Colorimetric) is tested against predetermined specifications to ensure consistent product quality. Epigentek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: Epigentek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Thus, only use the User Guide that was supplied with the kit when using that kit.

Usage Limitation: The EpiQuik™ BRD2 Binding Activity/Inhibition Assay Kit (Colorimetric) is for research use only and is not intended for diagnostic or therapeutic applications.

Intellectual Property: The EpiQuik™ BRD2 Binding Activity/Inhibition Assay Kit (Colorimetric) and methods of use contain proprietary technologies by Epigentek.

A BRIEF OVERVIEW

Bromodomains (BRDs), which are a diverse family of evolutionarily conserved protein-interaction modules, are found in histone acetyl transferases and other chromatin-associated proteins. More than 60 BRDs have been identified, which in turn cluster into eight families based on structure/sequence similarity. BRDs bind selectively to acetylated lysines, acting as "readers" of the histone code, and have recently been shown to regulate transcription activity; they also contain a druggable binding pocket. Proteins that contain BRD2 have been implicated in the development of a large variety of diseases and inhibitors that target BRDs of the BET (Bromodomains and extra-terminal), which inhibit BRD-mediated protein-protein interaction and have the potential to modulate multiple diseases including inflammation and cancer.

There are currently no conveniet methods used for detecting BRD2 binding activity/inhibition. Bead-based prepcipitation requires electrophoresis and immunoblotting processes, which make the assay inconvenient, time consuming, and low throughput. The FRET-based methods such as TR-FRET and

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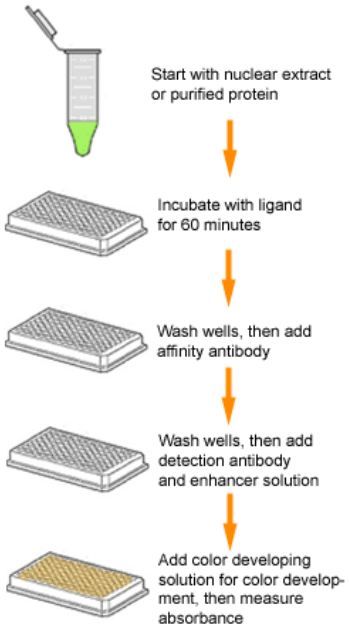
AlphaLISA allow for high throughput assays but require expensive equipment and purified proteins. The EpiQuik™ BRD2 Binding Activity/Inhibition Assay Kit addresses these problems by using a unique procedure to measure BRD2 binding activity/inhibition. The kit has the following features:

- Fast procedure, which can be finished within 6 hours.
- Innovative colorimetric assay which directly measures BRD2 binding activity in a 96-well plate using a standard microplate reade without the need for special reagents or expensive equipment.
- Both cell/tissue extracts and purified BRD2 protein can be used, which allows for the detection of inhibitory effects of BRD2 inhibitors in vivo and in vitro.
- High sensitivity and specificity – BRD2 binding-specific detection with a detection limit as low as 0.1 ng/well bound BRD2 protein.
- Strip microplate format makes the assay flexible via manual or high throughput analysis.
- BRD2 control is included, which allows the binding activity of BRD2 protein in the sample to be properly quantified.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

In an assay with this kit, the unique BRD2 ligand is stably coated onto the strip well. The sample is added into the well and BRD2 proteins contained in the sample bind to the ligand. The bound BRD2 can be recognized with a BRD2-specific antibody and colorimetrically quantified through an ELISA-like reaction. The amount of bound BRD2 is proportional to the intensity of color development.


Start with nuclear extract or purified protein
Incubate with ligand for 60 minutes
Wash wells, then add affinity antibody
Wash wells, then add detection antibody and enhancer solution
Add color developing solution for color development, then measure absorbance

Schematic procedure of the EpiQuik™ BRD2 Binding Activity/Inhibition Assay Kit (Colorimetric)

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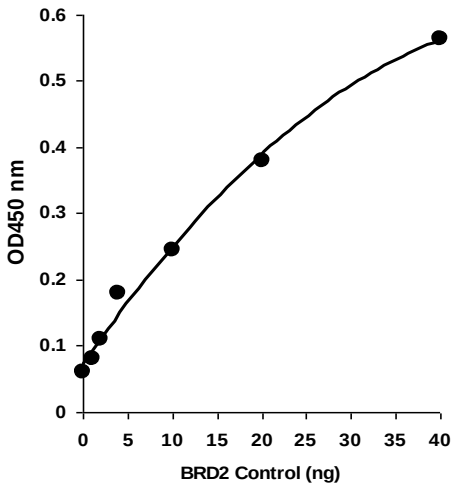
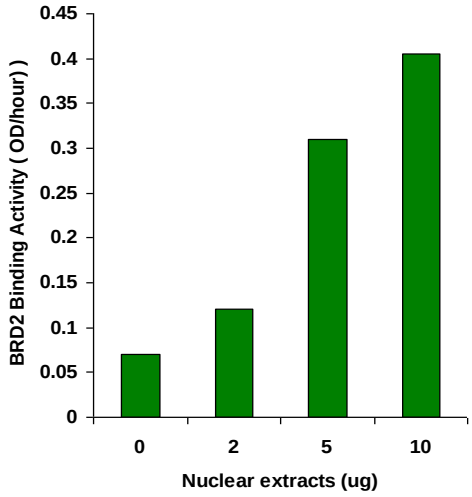


Illustrated standard curve generated with BRD2 control



Nuclear extracts were prepared from HeLa cells and the ODs generated from BRD2 are measured.

ASSAY PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

Starting Materials

Input Amount: The amount of nuclear extracts for each assay can be 1 µg to 20 µg with an optimized range of 5-10 µg. The amount of purified protein can be 10 ng to 500 ng, depending on the purity of the protein.

Nuclear Extraction: You can use your own successful method for preparing nuclear extracts from treated or untreated samples. Epigentek also offers a nuclear extraction kit (Cat # OP-0002) which has been optimized for use with this kit (see "Related Products" section).

Nuclear extracts should be stored at –80°C in aliquots until use.

1. Working Buffer and Solution Preparation

a. Prepare **Diluted WB** 1X Wash Buffer:

48-Assay Kit: Add 13 ml of **WB** 10X Wash Buffer to 117 ml of distilled water and adjust pH to 7.2-7.5.

96-Assay Kit: Add 26 ml of **WB** 10X Wash Buffer to 234 ml of distilled water and adjust pH to 7.2-7.5.

This **Diluted WB** 1X Wash Buffer can now be stored at 4°C for up to six months.

b. Prepare **1X BRL** BRD2 Ligand Solution:

Add 1 µl of **BRL** 100X BRD2 Ligand to 100 µl of **BRS** BRD2 Ligand Binding Solution. 100 µl of **1X BRL** will be required for each assay well.

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c. Prepare **Diluted CA** Capture Antibody Solution:

Dilute **CA** Capture Antibody with **Diluted WB** 1X Wash Buffer at a ratio of 1:1000 (i.e., add 1 µl of **CA** to 1000 µl of **Diluted WB**). 50 µl of **Diluted CA** will be required for each assay well.

d. Prepare **Diluted DA** Detection Antibody Solution:

Dilute **DA** Detection Antibody with **Diluted WB** 1X Wash Buffer at a ratio of 1:2000 (i.e., add 1 µl of **DA** to 2000 µl of **Diluted WB**). 50 µl of **Diluted DA** will be required for each assay well.

e. Prepare **Diluted ES** Enhancer Solution:

Dilute **ES** Enhancer Solution with **Diluted WB** at a ratio of 1:5000 (i.e., add 1 µl of **ES** to 5000 µl of **Diluted WB**). About 50 µl of this **Diluted ES** will be required for each assay well.

f. Prepare **Diluted BRD2 Control Standard**:

Suggested Standard Curve Preparation: First, dilute **BRD2 control** with **AB** to a concentration of 40 ng/µl by adding 2 µl of **BRD2 control** to 8 µl of **AB** Then, further prepare concentration points of 2, 4, 10, 20 and 40 ng/µl according to the following chart:

Tube	BRD2 (40 ng/µl)	AB	Resulting BRD2 Concentration
1	0.5 µl	9.5 µl	2 ng/µl
2	0.5 µl	4.5 µl	4 ng/µl
3	1.0 µl	3.0 µl	10 ng/µl
4	2.0 µl	2.0 µl	20 ng/µl
5	4.0 µl	0.0 µl	40 ng/µl

*Note: Keep each of the diluted solutions except **WB** 1X Wash Buffer on ice until use. Any remaining diluted solutions other than **Diluted WB** should be discarded if not used within the same day. The lower concentration point (ex: 0.5 ng/µl) can be also added if needed.*

2. BRD2 Binding/Inhibition Reaction

a. Predetermine the number of strip wells required for your experiment. It is advised to run replicate samples (include blank and positive controls) to ensure that the signal generated is validated. Carefully remove un-needed strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C).

b. Add 100 µl of **1X BRL** to each well. Ensure the solution coats the bottom of the well evenly.

c. Cover strip plate with plate seal or Parafilm M and incubate at 37°C for 120 min.

d. Remove the **1X BRL** from each well. Add 150 µl of **BB** Blocking Buffer to each well, then cover with Parafilm M or aluminum foil and incubate at 37°C for 30 min.

e. Wash each well three times with 150 µl of the **Diluted WB** 1X Wash Buffer each time.

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f. Blank Wells: Add 100 µl of **AB** to each blank well.

g. Standard Wells: Add 49 µl of **AB** and 1 µl of **Diluted BRD2 Control Standard** to each standard well, to a minimum of five wells, each at different concentrations between 1 and 20 ng/µl (based on the dilution chart in Step 1f; see Table 2 in the "Suggested Strip Well Setup" section as an example).

h. Sample Wells Without Inhibitor: Add 45 to 48 µl of **AB**, 2 to 5 µl of your nuclear extracts or 2 to 5 µl of your purified BRD2 protein. Total volume should be 50 µl per well.

i. Sample Wells With Inhibitor: Add 40 to 43 µl of **AB**, 2 to 5 µl of your nuclear extracts or 2 to 5 µl of purified BRD2 protein, and 5 µl of inhibitor solution. Total volume should be 50 µl per well.

*Note: (1) Follow the diagrams under the "Suggested Working Buffer & Solution Setup" section; (2) It is recommended to use 2 µg to 10 µg of nuclear extract per well or 20 ng to 100 ng of purified protein per well; (3) The concentration of inhibitor to be added into the sample wells can be varied (ex: 1 µM to 1000 µM). However, the final concentration of the inhibitors before adding to the wells should be prepared with **AB** at a 1:10 ratio (ex: add 0.5 µl of inhibitor to 4.5 µl of **AB**) so that the original solvent of the inhibitor can be reduced to 1% of the reaction solution or less. The BRD inhibitor, JQ1included in the kit can be used as the control inhibitor.*

j. Tightly cover strip plate with **Adhesive Covering Film** to avoid evaporation and incubate at 37°C for 60-120 min.

*Note: (1) The incubation time may depend on intrinsic BRD2 protein binding activity. However, in general, 60-90 min incubation is suitable for active purified proteins and 90-120 min incubation is suitable for nuclear extracts; (2) The **Adhesive Covering Film** can be cut to the required size to cover the strips based on the number of strips to be used.*

k. Remove the reaction solution from each well. Wash each well three times with 150 µl of the **Diluted WB** 1X Wash Buffer each time.

l. Remove the reaction solution from each well. Wash each well three times with 150 µl of the **Diluted WB** 1X Wash Buffer each time.

3. Antibody Binding & Signal Enhancing

a. Add 50 µl of the **Diluted CA** to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 60 min.

b. Remove the **Diluted CA** solution from each well.

c. Wash each well three times with 150 µl of the **Diluted WB** each time.

d. Add 50 µl of the **Diluted DA** to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 30 min.

e. Remove the **Diluted DA** solution from each well.

f. Wash each well four times with 150 µl of the **Diluted WB** each time.

g. Add 50 µl of the **Diluted ES** to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 30 min.

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h. Remove the **Diluted ES** solution from each well.

i. Wash each well five times with 150 µl of the **Diluted WB** each time.

Note: Ensure any residual wash buffer in the wells is removed as much as possible at each wash step.

4. Signal Detection

a. Add 100 µl of **DS** to each well and incubate at room temperature for 1 to 10 min away from light. Begin monitoring color change in the sample wells and control wells. The **DS** solution will turn blue in the presence of sufficient BRD2 protein.

b. Add 100 µl of **SS** to each well to stop enzyme reaction when color in the positive control wells turns medium blue. The color will change to yellow after adding **SS** and the absorbance should be read on a microplate reader within 2 to 10 min at 450 nm with an optional reference wavelength of 655 nm.

Note: (1) Most microplate readers have the capability to carry out dual wavelength analysis and will automatically subtract reference wavelength absorbance from the test wavelength absorbance. If your plate reader does not have this capability, the plate can be read twice, once at 450 nm and once at 655 nm. Then, manually subtract the 655 nm ODs from 450 nm ODs; (2) If the strip-well microplate frame does not fit in the microplate reader, transfer the solution to a standard 96-well microplate.

5. BRD2 Calculation

a. Calculate the average replicate readings for the sample wells and blank wells.
b. Calculate BRD2 binding activity or inhibition using the following formulas:

For simple calculation:

$$\textit{BRD2 Binding Activity (OD/min/mg)} = \frac{\textit{(Sample OD – Blank OD)}}{\textit{(Protein Amount (µg)* x min**)}} \textbf{ x } \textit{1000}$$

* Protein amount (µg) added into the reaction at step 2h.

** Incubation time (minutes) at step 2j.

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Example calculation:

Average OD450 of sample is 0.35
Average OD450 of blank is 0.05
Protein amount is 5 µg
Incubation time is 60 minutes (1 hour)
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$$\text{BRD2 Binding activity} = \frac{(0.35 - 0.05)}{(5 \times 60)} \times 1000 = 1 \text{ OD/min/mg}$$

For accurate binding activity calculation:

1. Generate a standard curve and plot OD value versus amount of **BRD2 Control Standard** at each concentration point.

2. Determine the slope as OD/ng (you can use Microsoft Excel statistical functions for slope calculation), then calculate the amount of bound BRD2 using the following formulas:

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$$BRD2\ (ng) = \frac{(Sample\ OD - Blank\ OD)}{Slope}$$

$$BRD2\ Binding\ Activity\ (ng/min/mg) = \frac{BRD2\ (ng)}{(Protein\ Amount\ (\mu g) \times min^*)} \times 1000$$

* Incubation time (minutes) at Step 2j.

For inhibition calculation:

$$Inhibition\ \% = \left[1 - \frac{Inhibitor\ Sample\ OD - Blank\ OD}{No\ Inhibitor\ Sample\ OD - Blank\ OD} \right] \times 100\%$$

SUGGESTED BUFFER AND SOLUTION SETUP

Table 1. Approximate amount of required buffers and solutions for defined assay wells based on the protocol.

Reagents	1 well	1 strip (8 wells)	2 strips (16 wells)	6 strips (48 wells)	12 strips (96 wells)
Diluted WB	2.5 ml	20 ml	40 ml	120 ml	240 ml
BRL	1 µl	8 µl	16 µl	48 µl	96 µl
BRS	100 µl	800 µl	1600 µl	4800 µl	9600 µl
AB	50 µl	400 µl	800 µl	2400 µl	4800 µl
BB	0.15 ml	1.2 ml	2.5 ml	7.5 ml	14.5 ml
BRD2 control	N/A	N/A	2 µL (optional)	4 µl	4 µl
BRD2 Inhibitor	N/A	N/A	5 µL	10 µL	20 µL
Diluted CA	50 µl	400 µl	800 µl	2400 µl	4800 µl
Diluted DA	50 µl	400 µl	800 µl	2400 µl	4800 µl
Diluted ES	50 µl	400 µl	800 µl	2400 µl	4800 µl
DS	0.1 ml	0.8 ml	1.6 ml	4.8 ml	9.6 ml
SS	0.1 ml	0.8 ml	1.6 ml	4.8 ml	9.6 ml

SUGGESTED STRIP WELL SETUP

Table 2. The suggested strip-well plate setup for BRD2 assay in a 48-assay format (in a 96-assay format, Strips 7 to 12 can be configured as Sample). The controls and samples can be measured in duplicate.

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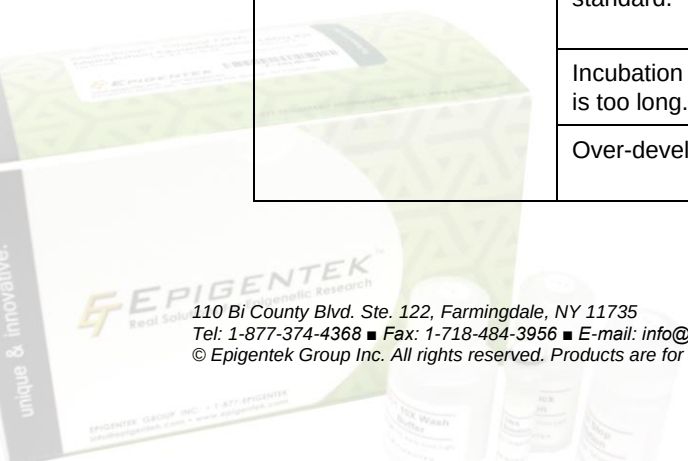
Well #	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	Blank	Blank	Sample	Sample	Sample	Sample
B	BRD2 2 ng	BRD2 2 ng	Sample	Sample	Sample	Sample
C	BRD2 4 ng	BRD2 4 ng	Sample	Sample	Sample	Sample
D	BRD2 10 ng	BRD2 10 ng	Sample	Sample	Sample	Sample
E	BRD2 20 ng	BRD2 20 ng	Sample	Sample	Sample	Sample
F	BRD2 40 ng	BRD2 40 ng	Sample	Sample	Sample	Sample
G	Sample	Sample	Sample	Sample	Sample	Sample
H	Sample	Sample	Sample	Sample	Sample	Sample

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
No signal or weak signal in both the positive control and sample wells	Reagents are added incorrectly.	Check if reagents are added in the proper order with the right amount, and if any steps in the protocol may have been omitted by mistake.
	Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.
	Incorrect absorbance reading.	Check if appropriate absorbance wavelength (450 nm) is used.
	Kit was not stored or handled properly.	Ensure all components of the kit were stored at the appropriate temperature and the cap is tightly capped after each opening or use.
No signal or weak signal in only the standard curve wells	The standard amount is insufficiently added to the well in Step 2g.	Ensure a sufficient amount of standard is added.
	The standard is degraded due to improper storage conditions.	Follow the Shipping & Storage guidance in this User Guide for storage of **BRD2 Control.**
High background present in the blank wells	Insufficient washing of wells.	Check if washing recommendations at each step is performed according to the protocol.
	Contaminated by sample or standard.	Ensure the well is not contaminated from adding sample or standard accidentally or from using contaminated tips.
	Incubation time with **Diluted DA** is too long.	The incubation time at Step 3d should not exceed 90 min.
	Over-development of color.	Decrease the development time in Step 4a before adding **SS** Stop Solution in Step 4b.



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No signal or weak signal only in sample wells	Protein sample is not properly extracted or purified.	Ensure your protocol is suitable for histone protein extraction. For the best results, it is advised to use Epigentek's Nuclear extraction Kit (Cat. No. OP-0002).
	Sample amount added into the wells is insufficient.	Ensure a sufficient amount of nuclear extracts is used as indicated in Step 2. The sample can be titrated to determine the optimal amount to use in the assay.
	Sample was not stored properly or has been stored for too long.	Ensure sample is stored in aliquots at – 80°C, with no more than 6 months nuclear extracts.
	Little or no BRD2 in the sample.	This problem may be a result of many factors. If the affecting factors cannot be determined, use new or re-prepared nuclear extracts.
Uneven color development	Insufficient washing of the wells.	Ensure the wells are washed according to the guidance of washing and residue washing buffer is removed as much as possible.
	Delayed color development or delayed stopping of color development in the wells.	Ensure color development solution or stop solution is added sequentially and is consistent with the order you added the other reagents (e.g., from well A to well G or from well 1 to well 12).

RELATED PRODUCTS

Nuclear Extract Preparation

OP-0002 EpiQuik™ Nuclear Extraction Kit

Histone Deacetylase Activity/Inhibition Assay

P-4034 Epigenase™ HDAC Activity/Inhibition Direct Assay Kit
P-4035 Epigenase™ HDAC Activity/Inhibition Direct Assay Kit (Fluorometric)
P-4036 Epigenase™ Universal SIRTActivity/Inhibition Assay Kit
P-4037 Epigenase™ Universal SIRT Activity/Inhibition Assay Kit (Fluorometric)
P-4003 EpiQuik™ HAT Activity/Inhibition Assay Kit

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